N-Acetyl-β-hexosaminidase: Role in the Degradation of Glycosaminoglycans

Abstract. *Extracts of cultured normal human skin fibroblasts released radioactivity from a ^{14}C-labeled heptasaccharide prepared by addition of [^{14}C]N-acetylgalactosamine to the nonreducing terminus of a hexasaccharide derived from chondroitin 4-sulfate whereas fibroblast extracts from patients with Tay-Sachs and Sandhoff-Jatzkewitz diseases did not. The results suggest that N-acetyl-β-hexosaminidase A is responsible for degradation of the oligosaccharide substrate.*

The importance of the specific action of glycosidases for maintenance of normal cell architecture and metabolism is indicated by the existence of heritable human diseases characterized by absence of specific glycosidase activities. The three major classes of macromolecules of eukaryotic cells that contain amino sugars—proteoglycans, glycoproteins, and glycosphingolipids—have certain glycosidic linkages in common. The enzymic defects in a number of glycosphingolipidoses (*1*) and mucopolysaccharidoses have now been discovered (*2*).

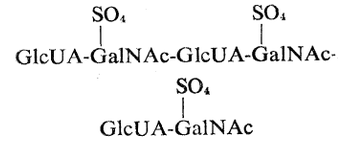
Glycosidases have been thought to be specific with respect to the structure of the glycone and the anomeric configuration of the glycoside bond, but nonspecific with respect to the adjacent portion of the molecules. *N*-Acetyl-β-hexosaminidase apparently is an important exception in that most studies indicate a lack of specificity with respect to the configuration at C-4 and a high degree of specificity of its isozymes toward the aglycone portion of the molecule. The degradation of the glycosaminoglycans has been considered to involve the *N*-acetyl-β-hexosaminidase present in a large number of mammalian tissues (*3*). This enzyme occurs in most tissues in at least two forms, which may be distinguished by isoelectric point and heat stability (*4*). Okada and O'Brien (*5*) showed that an absence of one of these forms, *N*-acetyl-β-hexosaminidase A is responsible for accumulation of G_{M2} ganglioside [GalNAc-(NeuNAc)-Gal-Glc-Cer] (*6*) in the nervous system in Tay-Sachs disease.

In Sandhoff-Jatzkewitz disease there is an almost complete absence of *N*-acetyl-β-hexosaminidase activity and an accumulation of G_{M2} ganglioside in the brain and of globoside (GalNAc-Gal-Gal-Glc-Cer) and asialo-G_{M2} in the kidneys (*7*). Since Sandhoff-Jatzkewitz disease appears to be autosomal recessive, it might be expected that both *N*-acetyl-β-hexosaminidase A and B are produced by the same structural gene. This is supported by the finding of Robinson and Stirling of conversion of hexosaminidase A to hexosaminidase B by the action of neuraminidase (*4*) and the report that hexosaminidase A and B cross react immunologically (*8*). Since absence of hexosaminidase A is sufficient to impair glycosphingolipid metabolism (Tay-Sachs disease), it must be assumed that the two forms of the enzyme have different specificities, although their structural relationship is not yet clear.

If *N*-acetyl-β-hexosaminidase A is obligatory for the degradation of glycosaminoglycans, such compounds should accumulate in Sandhoff-Jatzkewitz disease and in Tay-Sachs disease. However, neither Tay-Sachs disease nor Sandhoff-Jatzkewitz disease are clinically similar to the mucopolysaccharidoses. Strecker and Montreuil (*9*) were unable to find increased amounts of glycosaminoglycans in urines of either Tay-Sachs or Sandhoff-Jatzkewitz patients although increased quantities of what appeared to be oligosaccharides derived from glycoproteins were found in the urine of a patient with Sandhoff-Jatzkewitz disease.

In our experiments, the hydrolytic activity of extracts derived from cultured fibroblasts of patients with a number of storage diseases was tested with the use of a specifically labeled oligosaccharide related to chondroitin sulfate.

Fibroblast cultures were established as described (*10*). The medium was removed and the cells were washed with 0.05*M* sodium acetate–0.15*M* NaCl buffer (*p*H 5.5); the cells were then harvested with a rubber policeman, suspended in the same buffer, and disrupted with a Branson sonifier (approximately 1 minute with a microtip probe at 100 watts). The suspension was centrifuged at 10,000*g* for 10 minutes, and the supernatant was used as an enzyme source. Protein was determined by the method of Lowry *et al.* (*11*). Hexasaccharide obtained by the digestion of chondroitin 4-sulfate with testicular hyaluronidase was prepared according to Telser *et al.* (*12*) and has the following structure



A heptasaccharide specifically labeled in the nonreducing terminal GalNAc group was prepared by the transfer of [^{14}C]GalNAc from UDP-[^{14}C]GalNAc (51.5 mc/mmole) to 1.5 mg of chondroitin 4-sulfate hexasaccharide (molecular weight, 1400) with the use of particulate enzyme sedimenting at 105,000*g* and obtained from a homogenate of 13-day-old chick tibiae and femora epiphyses (*13*). The labeled reaction product was isolated by gel filtration on a column (1.1 by 200 cm) of Sephadex G-25 with 0.05*M* NaCl in 15 percent ethanol as eluant. The fractions containing [^{14}C]heptasaccharide (785,000 count/min) were pooled (20 to 25 ml), partially desalted by dialysis against six 100-ml portions of distilled water for 90 minutes, and lyophilized. For enzyme studies, fibroblast extracts

Table 1. Reactions of *N*-acetyl-β-hexosaminidase from extracts of cultured skin fibroblasts, measured as [^{14}C]*N*-acetylgalactosamine release (counts per minute per milligram of protein per 24 hours) or as 4-methylumbelliferone from umbelliferyl-*N*-acetyl-β-D-galactosaminide (micromoles per milligram of protein per hour). N.D., not detectable.

Enzyme source	[^{14}C]GalNAc (count/min)	4-Methylumbelliferone (μmole/mg)
Normal-1	5135	
Normal-2	5125	0.83
Hurler	5433	
Hunter	4652	
Sanfilippo	3577	
"I-Cell"	473	
Tay-Sachs	N.D.	0.24
Sandhoff-Jatzkewitz	N.D.	0.004
Normal-heat inactivated*	370	
Normal + Tay-Sachs	3738	
Normal + Sandhoff-Jatzkewitz	2880	
Sandhoff-Jatzkewitz + Tay-Sachs	N.D.	
Normal + "I-Cell"	2762	
"I-Cell" + Sandhoff-Jatzkewitz	89	

* The enzyme extract was heated at 50°C for 2 hours.

(0.2 to 0.4 mg of protein) were incubated with [^{14}C]heptasaccharide (20,000 count/min) in 0.10*M* sodium acetate–0.15*M* NaCl buffer, *p*H 4.5, in a final volume of 0.5 ml. Assays in a citrate buffer showed similar results. After incubation at 37°C for 24 hours (thymol was added to inhibit bacterial growth), reactions were stopped by heat (boiling water bath for 2 minutes). Supernatants containing the products were obtained by centrifugation at 250*g* for 5 minutes, and the products were separated by Sephadex gel filtration (see Fig. 1). *N*-Acetyl-*β*-hexosaminidase activity toward 4-methylumbelliferyl-*N*-acetyl-*β*-D-galactosaminide was measured (*14*).

Contrasting results were obtained with extracts of cultured fibroblasts from normal individuals and patients with Tay-Sachs and Sandhoff-Jatzkewitz diseases (Fig. 1). The reaction rate was linear with protein concentration and time of incubation, up to 24 hours; but it decreased slightly between 24 and 72 hours. After 72 hours, more than 75 percent of the substrate was hydrolyzed by extracts from control subjects. A sharp optimum was found at *p*H 4.5. Table 1 indicates the activity of various fibroblast extracts toward the glycosaminoglycan-oligosaccharide substrate compared, in some cases, with the activity toward the umbelliferyl substrate. Although Tay-Sachs extracts hydrolyzed the synthetic *N*-acetyl-*β*-glucosaminide substrate, they were completely inactive toward the [^{14}C]heptasaccharide to the extent that the release of as little as 0.2 percent of the radioactivity would have been detected. In a separate experiment extracts of Tay-Sachs and Sandhoff-Jatzkewitz fibroblasts which showed no activity toward the oligosaccharide were shown to hydrolyze *p*-nitrophenyl-*N*-acetyl-*β*-D-galactosaminide. Mixtures of cell extracts from normal fibroblasts and "I-cell" or Sandhoff-Jatzkewitz cells had intermediate activity, but mixed extracts from normal and Tay-Sachs fibroblasts exhibited slightly higher activity than expected from theory. This result was found several times, but the explanation of the elevated activity has not been investigated. The low activity for "I-cell" extracts is in keeping with diminished activity of several glycosidases, including *N*-acetyl-*β*-hexosaminidase, as previously reported (*15*).

The complete inactivity of Tay-Sachs and Sandhoff-Jatzkewitz extracts, as well as the minimal activity of heat-inactivated extracts, suggests that *N*-acetyl-*β*-hexosaminidase B is not involved in the degradation of the glycosaminogylcan oligosaccharide. A difference in specificity of the two forms of *N*-acetyl-*β*-hexosaminidase with respect to ganglioside has already been observed (*5*, *16*).

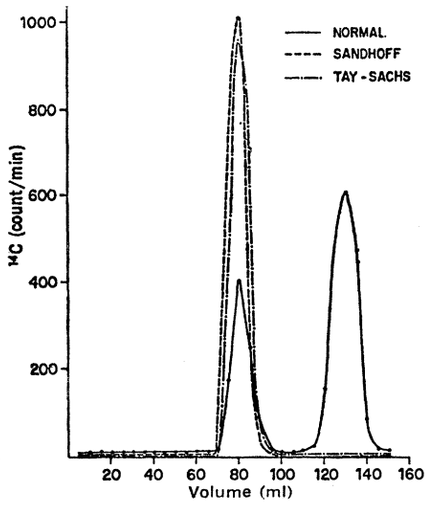


Fig. 1. Separation of reaction products by Sephadex gel filtration. Reaction products in supernatant were transferred onto columns (1.1 by 200 cm) of Sephadex G-25 and eluted with 0.05*M* NaCl in 15 percent ethanol. The [^{14}C]heptasaccharide (first peak) and released [^{14}C]GalNAc (second peak) were clearly resolved. The column was standardized with authentic [^{14}C]GlcNAc. Radioactivity was measured in a liquid scintillation spectrometer as described (*20*).

Since Tay-Sachs and Sandhoff-Jatzkewitz diseases are not characterized by glycosaminoglycan deposition, these results raise an important question regarding the normal pathway of degradation of glycosaminoglycans. Hyaluronic acid and chondroitin 4- and 6-sulfates may be partially degraded by hyaluronidase to yield oligosaccharides. Complete degradation, however, requires the action of exoglycosidases. Matalon and Dorfman (*17*) have been unable to demonstrate hyaluronidase activity in fibroblast extracts and have suggested that glycosaminoglycan degradation in fibroblasts occurs primarily by stepwise action of exoglycosidases.

Frohwein and Gatt (*18*) have suggested that calf brain contains *N*-acetyl-*β*-hexosaminidases specific for degradation of *N*-acetylglucosaminides and *N*-acetylgalactosaminides which differ from the more frequently studied hexosaminidases that are nonspecific for the anomeric configuration at C-4. Robinson *et al.* (*19*) found no evidence for such enzymes in calf brain.

Our data indicate another example of the specificity of the isozymes of *N*-acetyl-*β*-hexosaminidase and describe an easily prepared substrate for *N*-acetyl-*β*-hexosaminidase A, which may be useful for the diagnosis of Tay-Sachs disease. Since this enzyme activity is absent in Tay-Sachs and Sandhoff-Jatzkewitz diseases, the lack of glycosaminoglycan accumulation in these diseases remains unexplained.

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6. Abbreviations: Gal, galactose; GalNAc, *N*-acetylgalactosamine; Glc, glucose; Cer, ceramide; GlcUA, glucuronic acid; NeuNAc, *N*-acetylneuraminic acid; UDP, uridine diphosphate.
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